Electronic Supplementary Material (ESI) for Chemical Communications

Direct and selective tagging of cysteine residues in peptides and proteins with 4-nitropyridyl lanthanide complexes

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Contents:

1. Experimental Section

General. Chemicals were purchased from commercial suppliers (Aldrich, Fluka, Merck) and were used without further purification unless otherwise stated. Peptides were purchased from Peptide Protein Research Ltd. Solvents were dried using an appropriate drying agent when required (CH_3CN over CaH_2, CH_3OH over $Mg(OMe)_2$ and THF over Na/benzophenone). Unless and otherwise mentioned, reactions were carried out under an argon atmosphere and the reaction flasks were pre-dried under reduced pressure. Ultra pure de-ionised water (18 MΩ cm^{-1}) was used throughout. All glassware was washed with a mixed acid solution and thoroughly rinsed with de-ionized, distilled water.

Spectroscopy. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in commercially-available deuteriated solvents on a Varian Mercury-200 (^{1}H at 199.975 MHz, ^{13}C at 50.289 MHz, ^{19}F at 188.179 MHz), Varian Mercury-400 or Bruker Avance-400 (^{1}H at 399.960 MHz, ^{13}C at 100.572 MHz, ^{19}F at 376.338 MHz, ^{31}P at 161.943 MHz), Varian Inova-500 (^{1}H at 499.772 MHz, ^{13}C at 125.671 MHz, ^{19}F at 470.253 MHz) or Varian VNMRS-700 (^{1}H at 699.731 MHz, ^{19}F at 658.407 MHz, ^{31}P at 283.256 MHz) spectrometer. All chemical shifts are given in ppm and coupling constants are in Hz. Electrospray mass spectra were recorded on a Waters Micromass LCT or Thermo-Finnigan LTQ FT instrument operating in positive or negative ion mode as stated, with methanol as the carrier solvent. Accurate mass spectra were recorded using the Thermo-Finnigan LTQ FT mass spectrometer. LC-MS analyses were performed on a Waters system comprising a 3100 Mass Detector and a 2998 Photodiode array detector.

^{1}H NMR spectra of lanthanide complexes are assigned where possible and a spectral range has been quoted in each case; however, for some spectra a complete assignment was not feasible. For each complex spectrum, only the resonances of the major isomer are analysed.

Chromatography. Flash column chromatography was performed using flash silica gel 60 (230 - 400 mesh) from Merck. Thin layer chromatography (TLC) was performed on aluminium sheet silica gel plates with 0.2 mm thick silica gel 60 F_{254}

(E. Merck) using different mobile phase. The compounds were visualized by UV irradiation (254 nm).

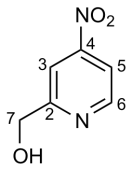
Reverse phase HPLC traces were recorded at 298 K using a Perkin Elmer system equipped with a Perkin Elmer Series 200 Pump, a Perkin Elmer Series 200 Autosampler and a Perkin Elmer Series 200 Diode array detector (operated at 254 nm). Separation was achieved using a semi-preparative Waters XBridge RP-C_{18} column (5 μm, 10 × 100 mm) at a flow rate maintained at 4.4 mL/min. A solvent system composed of H_2O + 0.1% HCOOH/methanol + 0.1% HCOOH was used over the stated linear gradient. Analytical RP-HPLC was performed using a Waters XBridge RP-C_{18} column (3.5 μm, 4.6 × 100 mm) at a flow rate maintained at 1.0 mL/ min over the stated linear gradient.

Optical Spectroscopy

UV/Vis absorbance spectra were recorded on a Perkin Elmer Lambda 900 UV/Vis/NIR spectrometer using FL Winlab software. Emission spectra were recorded on an ISA Joblin-Yvon Spex Fluorolog-3 luminescent spectrometer using DataMax v2.20 software. Lifetimes were measured on a Perkin Elmer LS55 luminescence spectrometer using FL Winlab Molecular Spectroscopy Version 4.00.02 software. Each sample was recorded in quartz cuvettes (path length = 1 cm) at 298 K. Generally, an integration time of 0.5 seconds, increment of 0.5 nm and excitation and emission slits of 2.5 and 1.5 nm respectively were used.

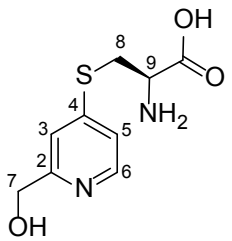
Ligand and Complex Synthesis

2-Hydroxymethyl-4-nitropyridine



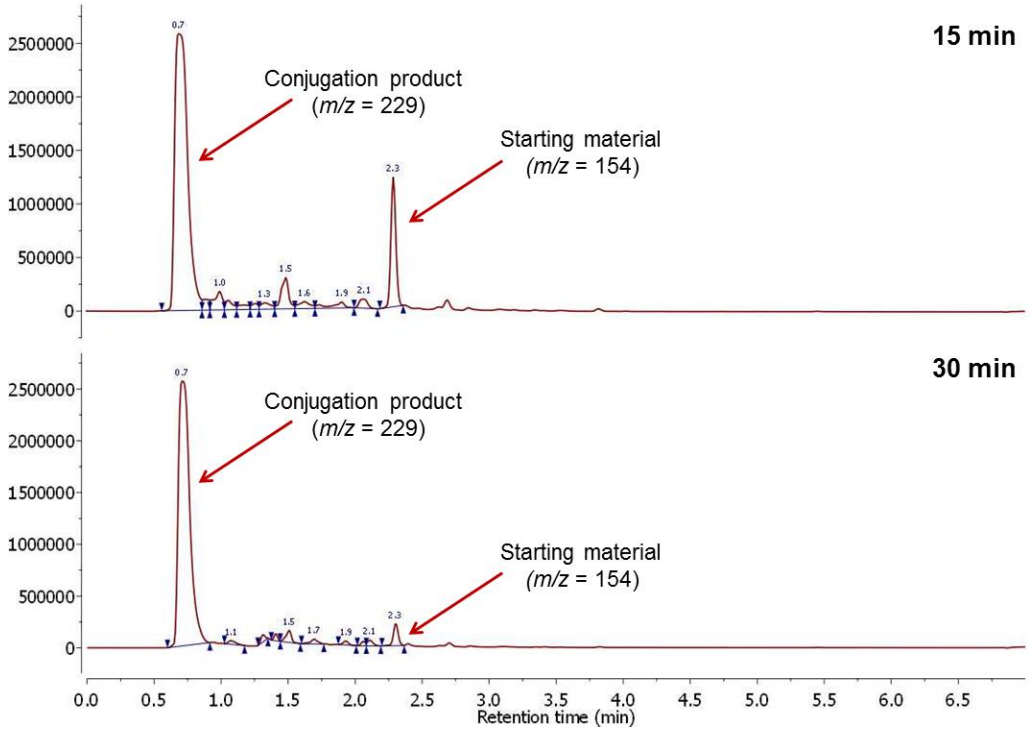
4-Nitro-2-picoline N-oxide (0.92 g, 6.0 mmol) was dissolved in CH_2Cl_2 (25 mL). A solution of trifluoroacetic anhydride (2.5 mL, 18 mmol) in CH_2Cl_2 (5 mL) was added dropwise and the resulting red solution stirred at r.t. for 3 days. Solvent was removed under reduced pressure to give a pale red oil. MeOH (20 mL) and saturated potassium carbonate solution (10 mL) were added and the mixture was stirred for 16 h. MeOH was removed under reduced pressure and the residue taken up into CH_2Cl_2 (3 x 25 mL). The combined organic layers were washed with brine (25 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the crude product was purified by silica gel column chromatography (gradient elution: CH_2Cl_2 to 3 % CH_3OH : CH_2Cl_2), to give a yellow solid (0.24 g, 27 %); m.p. 92–94 ^oC; ^1H NMR ($CDCl_3$, δ): 8.87 (1H, d, $^3J_{H\text{-}H}$ 5 Hz, H^6), 8.08 (1H, d, $^4J_{H\text{-}H}$ 2 Hz, H^3), 7.95 (1H, dd, $^3J_{H\text{-}H}$ 5 Hz, $^4J_{H\text{-}H}$ 2 Hz, H^5), 4.94 (2H, s, H^7), 2.70 (1H, br, OH); ^{13}C NMR ($CDCl_3$, *δ*): 163.4, 154.6, 151.1, 115.1, 113.3 (Ar), 64.5 (C^7); *m/z* (HRMS$^+$) 155.0449 $[M+H]^+$ ($C_6H_7O_3N_2$ requires 155.0457); R_f = 0.50 (10% CH_3OH : CH_2Cl_2).

2-Hydroxymethyl-4-(L-cysteinyl)pyridine



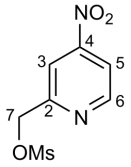
2-Hydroxymethyl-4-nitropyridine (20 mg, 0.130 mmol) was dissolved in H_2O (2.5 mL) and the pH of the solution was adjusted to 7.0 by the addition of KOH. L-Cysteine (16 mg, 0.130 mmol) was added and the mixture was stirred at r.t. for 30

min. The progress of the reaction was monitored by LC-MS, which revealed >93% conversion of starting material after 30 min (see below). The solvent was removed under reduced pressure to give the crude product as a yellow oil; ^{1}H NMR (D_2O, δ): 8.09 (1H, d, $^{3}J_{H\text{-}H}$ 5 Hz, H^{6}), 7.26 (1H, s, H^{3}), 7.12 (1H, d, $^{3}J_{H\text{-}H}$ 5 Hz, H^{5}), 4.50 (2H, s, H^{7}), 3.41 (1H, dd, $^{3}J_{H\text{-}H}$ 7 Hz, $^{3}J_{H\text{-}H}$ 5 Hz, H^{9}), 3.28 (1H, dd, $^{2}J_{H\text{-}H}$ 14 Hz, $^{3}J_{H\text{-}H}$ 5 Hz, H^{8}), 3.09 (1H, dd, $^{2}J_{H\text{-}H}$ 14 Hz, $^{3}J_{H\text{-}H}$ 7 Hz, H^{8}), N-H and O-H signals not observed; *m/z* (HRMS^{+}) 229.0640 $[M+H]^{+}$ ($C_9H_{13}O_3N_2S$ requires 229.0647).



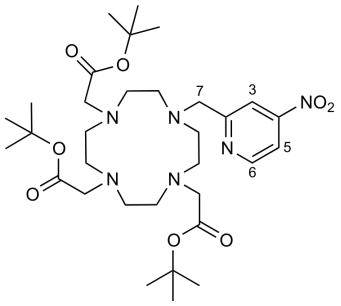
Analytical LC-MS chromatograms showing the formation of 2-hydroxymethyl-4-(L-cysteinyl)pyridine (and depletion of 2-hydroxymethyl-4-nitropyridine) over 30 min. t_R = 0.7 min [Gradient: 5 to 100% methanol in water (0.1% formic acid) over 5 min].

2-Methyl(sulfonyloxymethyl)-4-nitropyridine



2-Hydroxymethyl-4-nitropyridine (143 mg, 0.93 mmol) was dissolved in anhydrous THF. Triethylamine (388 μL, 2.78 mmol) was added and the mixture cooled to 0 ^{o}C under an inert atmosphere of $Ar_{(g)}$. Methanesulphonyl chloride (110 μL, 1.39 mmol) was added and the reaction mixture stirred for 15 min at 0 ^{o}C. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (20 mL) and washed with brine (20 mL). The aqueous layer was re-extracted into CH_2Cl_2 (2 x 15 mL), the organic layers dried over $MgSO_4$, and the solvent removed under reduced pressure to afford the product as a yellow oil (0.208 g, quantitative); ^{1}H NMR ($CDCl_3$, δ): 8.92 (1H, d, $^{3}J_{H\text{-}H}$ 5.2 Hz, H^{6}), 8.20 (1H, d, $^{4}J_{H\text{-}H}$ 2 Hz, H^{3}), 8.03 (1H, dd, $^{3}J_{H\text{-}H}$ 5.2 Hz, $^{4}J_{H\text{-}H}$ 2 Hz, H^{5}), 5.46 (2H, s, H^{7}), 3.18 (3H, s, Ms);^{13}C NMR ($CDCl_3$, δ): 157.5, 154.7, 152.0, 116.3, 114.7 (Ar), 69.8, 38.2; *m/z* (HRMS^{+}) 233.0232 $[M+H]^{+}$ ($C_7H_9N_2O_5S$ requires 233.0230); R_f = 0.76 (2 % CH_3OH : CH_2Cl_2).

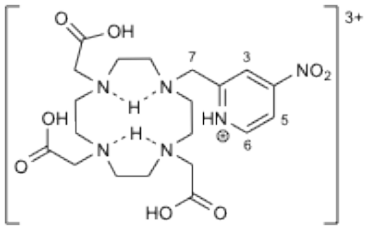
1-(4-Nitropyridyl-2-methyl)-4, 7, 10-tris(*tert*-butoxycarbonylmethyl)-1, 4, 7, 10-tetraazacyclododecane



2-Methyl(sulfonyloxymethyl)-4-nitropyridine (167 mg, 0.718 mmol), the macrocyclic tri-ester, DO3A^{t}Bu (370 mg, 0.718 mmol), and K_2CO_3 (99 mg, 0.718 mmol) were dissolved in anhydrous acetonitrile (40 mL) under an atmosphere of $Ar_{(g)}$ with stirring. The mixture was heated under reflux at 80^{o} C for 2 h. TLC was used to check for complete conversion (R_f = 0.13, 4 % CH_3OH: CH_2Cl_2). Solids were removed by

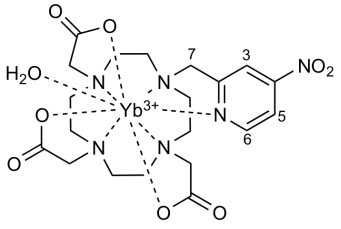
vacuum filtration and the solvent under reduced pressure. Purification by alumina gel column chromatography (gradient elution: CH_2Cl_2 to 3 % CH_3OH : CH_2Cl_2) afforded the product as a bright yellow oil (0.261 g, 56 %); ^{1}H NMR ($CDCl_3$, δ): 8.49 (1H, d, $^3J_{H\text{-}H}$ 3.6 Hz, H^6), 7.92 (1H, d, $^4J_{H\text{-}H}$ 1.5 Hz, H^3), 7.83 (1H, dd, $^3J_{H\text{-}H}$ 3.6 Hz, $^4J_{H\text{-}H}$ 1.5 Hz, H^5), 2.04 – 3.52 (24H, m), 1.12 – 1.48 (27H, m, 3 x ^{t}Bu); ^{13}C NMR ($CDCl_3$, δ): 172.7, 170.5, 162.8, 154.2, 150.75 (C^6), 115.9 (C^3), 114.7 (C^5), 82.1, 58.9, 58.0, 56.3 (br), 55.4, 47.9–52.8 (br), 28.1 (d), 27.9 (d); *m/z* (HRMS$^+$) 651.4060 $[M+H]^+$ ($C_{32}H_{55}O_8N_6$ requires 651.4076).

1-(4-Nitropyridyl-2-methyl)-4, 7, 10-tris(carboxymethyl)-1, 4, 7, 10-tetraazacyclododecane; $[H_3L^1]^{3+}$



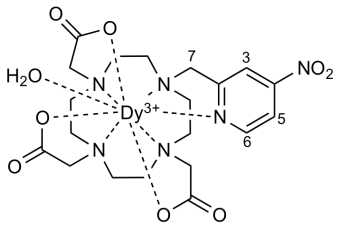
1-(4-Nitropyridyl-2-methyl)-4, 7, 10-tris(*tert*-butoxycarbonylmethyl)-1, 4, 7, 10-tetraazacyclododecane (50 mg, 0.08 mmol) was dissolved in CH_2Cl_2 (0.2 mL) and a drop of water added (0.1 mL). The reaction mixture was cooled in an ice bath, and TFA (1 mL) added slowly. The reaction mixture was allowed to warm to r.t. and was stirred for 24 h. Solvent was removed under reduced pressure to afford **L^1** as the tris(trifluoroacetate) salt, as an orange oil that was used directly for complexation (37 mg, quantitative); ^{1}H NMR (D_2O, δ): 8.76 (1H, d, $^3J_{H\text{-}H}$ 5.2 Hz, H^6), 8.20 (1H, s, H^3), 8.09 (1H, d, $^3J_{H\text{-}H}$ 5.2 Hz, H^5), 4.15 (2H, s, H^7), 2.81-3.72 (22H, m, $H^{9\text{-}20}$); *m/z* (HRMS$^+$) 483.2196 $[M+H]^+$ ($C_{20}H_{31}N_6O_8$ requires 483.2203).

$[Yb.L^1]$



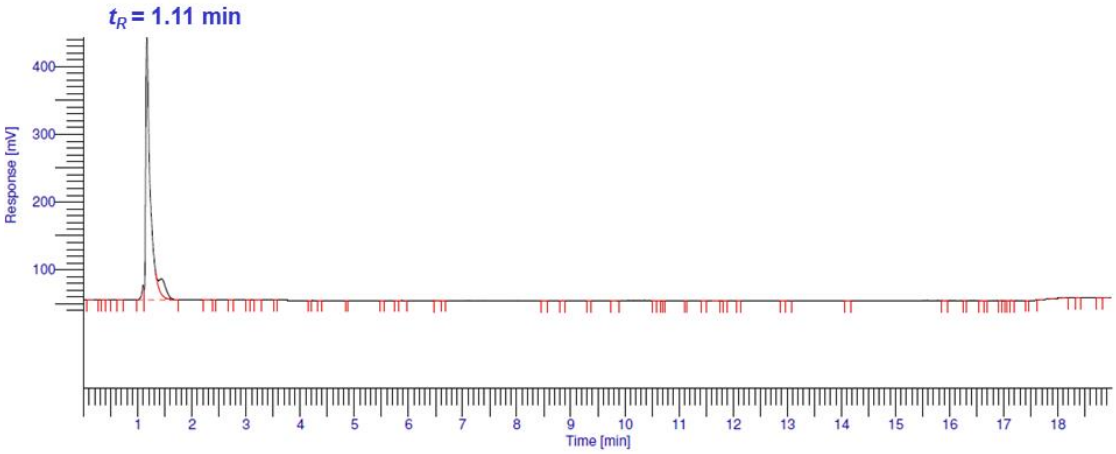
Ligand **L^1** (37 mg, 0.076 mmol) was dissolved in water (1 mL) and the pH adjusted to 6 with aqueous dilute NaOH solution. Ytterbium (III) chloride hexahydrate (32 mg, 0.084 mmol) was dissolved in water (1 mL) and added to the reaction mixture, maintaining the pH at 6. The reaction mixture was heated at 60 ^{o}C for 16 h. ESMS was used to verify complex formation. Excess Yb^{3+} was removed by raising the pH to 10 and filtering the solution. Solvent was removed under reduced pressure to afford the product as a clear yellow glassy solid (36 mg, 72 %); ^{1}H NMR ($CDCl_3$, δ): Spectral range of 204 ppm (+123 to -81 ppm), 122.2 (cyclen C$\underline{H}_{ax}$), 101.3 (cyclen C$^7\underline{H}_{ax}$), 101.0 (C$^7\underline{H}_2$), 83.1 (cyclen C$\underline{H}_{ax}$), 67.1 (cyclen C$\underline{H}_{ax}$), 48.8 (cyclen C$\underline{H}_{eq}$), 33.9 (cyclen C$\underline{H}_{eq}$), 22.2 (cyclen C$\underline{H}_{eq}$), 21.5 (cyclen C$\underline{H}_{eq}$), 19.5 (cyclen C$\underline{H}_{eq'}$), 16.8 (cyclen C$\underline{H}_{eq'}$), 11.5 (cyclen C$\underline{H}_{eq'}$), 6.8 (pyridyl C$\underline{H}$), 6.5 (pyridyl C$\underline{H}$), 6.2 (pyridyl C$\underline{H}$), 3.0 (cyclen C$\underline{H}_{eq'}$), -1.6 ("para" acetate C$\underline{H}_{ac'}$), -11.7 (acetate C$\underline{H}_{ac'}$), -14.7 (acetate C$\underline{H}_{ac'}$), -49.4 (acetate C$\underline{H}_{ac}$), -51.2 (acetate C$\underline{H}_{ac}$), -58.3 (cyclen C$\underline{H}_{ax'}$), -60.2 (cyclen C$\underline{H}_{ax'}$), -61.9 (cyclen C$\underline{H}_{ax'}$), -62.8 (C$^7\underline{H}_2$), -63.6 (cyclen C$\underline{H}_{ax'}$), -80.4 ("para" acetate C$\underline{H}_{ac}$); *m/z* (HRMS$^+$) 650.1319 $[M+H]^+$ ($C_{20}H_{28}N_6O_8$ ^{170}Yb requires 650.1316); λ_{max} (H_2O) = 298 nm.

[Dy.L^1]



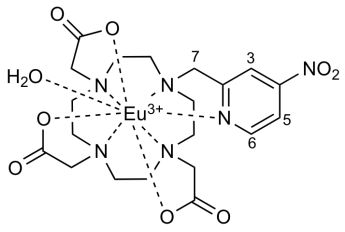
Complexation of ligand **L^1** (37 mg, 0.077 mmol) with Dy^{3+} using dysprosium (III) chloride hexahydrate (32 mg, 0.085 mmol) was undertaken as described above for complex [**Yb.L^1**]. The product was isolated as the tris(trifluoroacetate) salt, a pale yellow solid (50 mg, quantitative); decomposes above 165 ^{o}C; ^{1}H NMR (D_2O, δ): Spectral range of 970 ppm (+450 to -520 ppm), 287.7 (acetate C$\underline{H}_{ac}$), 277.5 (acetate C$\underline{H}_{ac}$), 21.4 (acetate C$\underline{H}_{ac'}$), 13.5 (acetate C$\underline{H}_{ac'}$), -15.4 (cyclen C$\underline{H}_{eq'}$), -38.3 (cyclen C$\underline{H}_{eq'}$), -46.3 (acetate C$\underline{H}_{ac}$), -69.4 (acetate C$\underline{H}_{ac'}$), -78.5 (cyclen C$\underline{H}_{eq'}$), -95.4 (cyclen C$\underline{H}_{eq'}$), -115.5 (cyclen C$\underline{H}_{eq}$), -166.1 (cyclen C$\underline{H}_{eq}$), -183 (cyclen C$\underline{H}_{eq}$), -276.3

(cyclen C<u>H</u>$_{eq}$); *m/z* (HRMS$^+$) 641.1250 $[M+H]^+$ ($C_{20}H_{28}N_6O_8{}^{161}Dy$ requires 641.1238); Analytical RP-HPLC: t_R = 1.11 min [gradient: 5 − 50% methanol in water (0.1% formic acid) over 15 min].

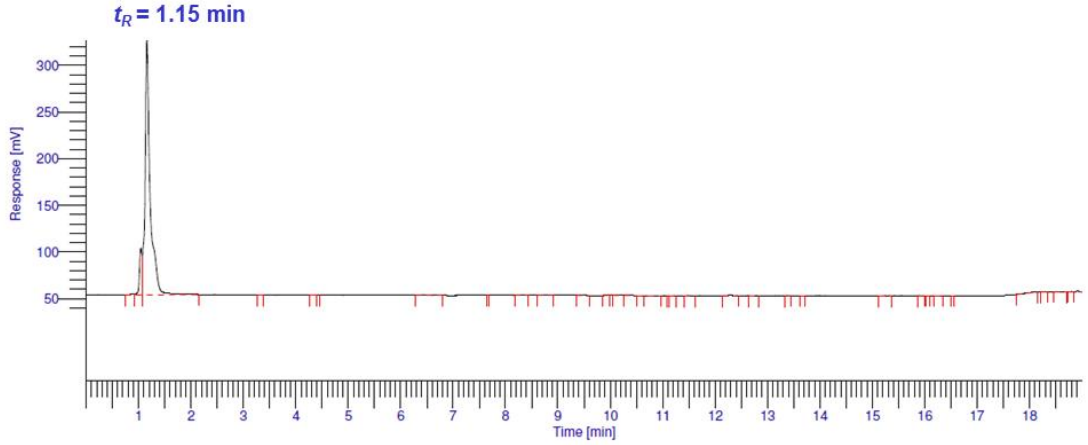


Analytical RP-HPLC trace of [**Dy.L^1**]: t_R 1.11 min [Gradient: 5 to 50% methanol in water (0.1% formic acid) over 15 min]

[Eu.L^1]

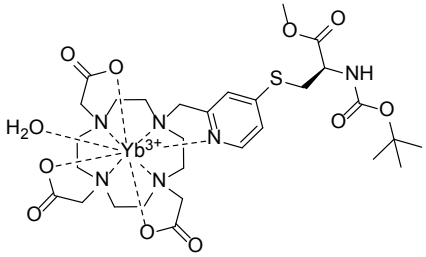


Complexation of ligand **L^1** (45 mg, 0.092 mmol) with Eu^{3+} using europium (III) chloride hexahydrate (37 mg, 0.101 mmol) was executed as above for complex [**Yb.L^1**]. The product was isolated as its tris(trifluoroacetate) salt, a yellow solid (106 mg, quantitative); decomposes above 200 ^oC; partial ^1H NMR (D_2O, δ): Spectral width of 57 ppm (+35 to -22 ppm), 33.2 (cyclen <u>H</u>$_{ax}$), 27.5 (2H, cyclen <u>H</u>$_{ax}$ & C^7<u>H</u>), 22.6 (cyclen <u>H</u>$_{ax}$), 19.7 (cyclen <u>H</u>$_{ax}$); m/z (HRMS$^+$) 631.1162 $[M+H]^+$ ($C_{20}H_{28}N_6O_8{}^{151}Eu$ requires 631.1167); λ_{max} (H_2O) = 298 nm; τ_{H2O} = 0.55 ms; τ_{D2O} = 1.40 ms, q = 0.9.



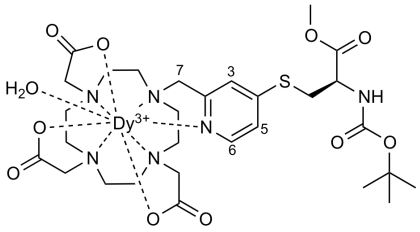
Analytical RP-HPLC trace of [**Eu.L^1**]: t_R 1.15 min [Gradient: 5 to 50% methanol in water (0.1% formic acid) over 15 min]

[Yb.L^1]-Boc-Cys-OMe



To a solution of [**Yb.L^1**] (10 mg, 0.015 mmol) in DMF (1 mL) was added *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (5 µL, 0.017 mmol) and DIPEA (4 µL, 0.030 mmol), and the mixture stirred at r.t. for 1 h. The solvent was removed under reduced pressure, and the residue was purified by reverse-phase preparative-HPLC (gradient: 10 – 100 % methanol in water over 20 min; t_R = 6.4 min) to give the Cys-bound Yb complex as a white solid (2 mg, 16 %); decomposes above 170 °C; ^{1}H NMR (D_2O, δ): 120.7 (cyclen C$\underline{H}_{ax}$), 112.0 (cyclen C$\underline{H}_{ax}$), 109.5 (cyclen C$\underline{H}_{ax}$), 95.3 (cyclen C$\underline{H}_{ax}$), 57.8 (cyclen C$\underline{H}_{eq}$), 40.0 (cyclen C$\underline{H}_{eq}$), 28.1 (cyclen C$\underline{H}_{eq}$), 19.8 (cyclen C$\underline{H}_{eq}$), 15.6 (cyclen C$\underline{H}_{eq'}$), 14.9 (cyclen C$\underline{H}_{eq'}$), 13.2 (cyclen C$\underline{H}_{eq'}$), 10.9 (cyclen C$\underline{H}_{eq'}$), multuplet including solvent resonance, -8.0 ("para" C$\underline{H}_{ac'}$), -19.2 (2H, acetate C$\underline{H}_{ac'}$), -46.1 (acetate C$\underline{H}_{ac}$), -46.5 (acetate C$\underline{H}_{ac}$), -54.0 ("para" acetate C$\underline{H}_{ac}$), -63.3 (cyclen C$\underline{H}_{ax'}$), -65.7 (cyclen C$\underline{H}_{ax'}$), -68.2 (cyclen C$\underline{H}_{ax'}$), -69.1 (C$^7\underline{H}$), -70.0 (cyclen C$\underline{H}_{ax'}$); *m/z* (HRMS$^+$) 838.2203 $[M+H]^+$ ($C_{29}H_{44}N_6O_{10}S$ ^{170}Yb requires 838.2187); λ_{max} (H_2O) = 280 nm.

[Dy.L^1]-Boc-Cys-OMe



Method 1

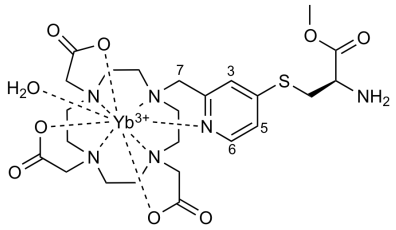
[**Dy.L^1**] (15 mg, 0.023 mmol) was dissolved in DMF (1 mL) and *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (5 µL, 0.025 mmol) and DIPEA (7 µL, 0.046 mmol) were added, and the mixture stirred at r.t. for 16 h. The solvent was removed under reduced pressure, and the residue was purified by reverse-phase preparative-HPLC (gradient: 10 – 100 % methanol in water over 20 mins; t_R = 9.2 min) to give the Cys-bound Dy complex as a white solid (10 mg, 52 %); decomposes above 185 ^oC; ^1H NMR (D_2O, δ): Spectral width of 885 ppm (+385 to -500 ppm), 382 (br), 343 (br), 328 (br), 266 (br), 250.3 (acetate C$\underline{H}_{ac}$), 240.6 (acetate C$\underline{H}_{ac}$), 232 (br), -38.2 (cyclen C$\underline{H}_{eq'}$), -47.3 (acetate C$\underline{H}_{ac}$), -68.9 (acetate C$\underline{H}_{ac'}$), -82.9 (cyclen C$\underline{H}_{eq'}$), -95.8 (cyclen C$\underline{H}_{eq'}$), -115.5 (cyclen C$\underline{H}_{eq}$), -161.0 (cyclen C$\underline{H}_{eq}$), -234.0, -403 (br), -413 (br), -499 (br); *m/z* (HRMS$^+$) 829.2098 $[M+H]^+$ ($C_{20}H_{28}N_6O_{10}$ ^{161}Dy requires 829.2075).

Method 2 (competition experiment)

To a solution of [**Dy.L^1**] (5 mg, 7.78 µmol) in DMF (1 mL) was added *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (1.6 µL, 7.78 µmol), *N*-(*tert*-butoxycarbonyl)-L-lysine (38 mg, 0.156 mmol) and DIPEA (2.7 µL, 15.6 µmol), and the mixture stirred at r.t. for 15 min. The reaction progress was monitored by LC-MS analysis, which revealed complete and selective conversion of the starting material to the conjugate, [**Dy.L^1**]-Boc-Cys-OMe, within 15 min. The solvent was removed under reduced pressure, and the residue was purified by reverse-phase preparative-HPLC (gradient: 10 – 100 % methanol in water over 20 mins; t_R = 9.2 min) to give [**Dy.L^1**]-

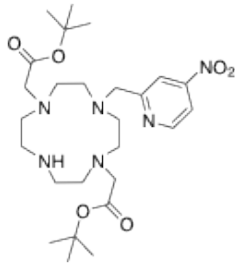
Boc-Cys-OMe as a white solid (3 mg, 46%). Characterisation data was identical to that described in Method 1.

[Yb.L^1]-Cys-OMe



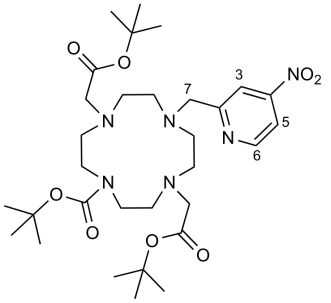
To a solution of [**Yb.L^1**] (10mg, 0.015 mmol) in DMF (1 mL) was added *N*-L-cysteine methyl ester (6 μL, 0.02 mmol) and DIPEA (4 μL, 0.03 mmol) and the mixture stirred at r.t. for 3 h. Solvent was removed under reduced pressure, and the product was isolated as a white solid (10 mg, 58 %); decomposes above 170 ^{o}C; ^{1}H NMR (D_2O, δ): Two isomeric species were observed in a 1:2 ratio, spectral range of 197 ppm (+124 to -73 ppm); 123.7 (cyclen C$\underline{H}_{ax}$), 113.6 (cyclen C$\underline{H}_{ax}$), 111.4 (cyclen C$\underline{H}_{ax}$), 96.7 (cyclen C$\underline{H}_{ax}$), 41.5 (cyclen C$\underline{H}_{eq}$), 29.0 (cyclen C$\underline{H}_{eq}$), 20.1 (cyclen C$\underline{H}_{eq'}$), 19.4 (cyclen C$\underline{H}_{eq}$), 16.3 (cyclen C$\underline{H}_{eq'}$), 14.9 (cyclen C$\underline{H}_{eq}$), 12.9 (cyclen C$\underline{H}_{eq'}$), 10.9 (cyclen C$\underline{H}_{eq'}$), multuplet including solvent resonance, -7.6 (“para” C$\underline{H}_{ac'}$), -19.5 (2H, acetate C$\underline{H}_{ac'}$), -47.6 (2H, acetate C$\underline{H}_{ac}$), -56.0 (“para” acetate C$\underline{H}_{ac}$), -64.7 (cyclen C$\underline{H}_{ax'}$), -66.9 (cyclen C$\underline{H}_{ax'}$), -69.5 (cyclen C$\underline{H}_{ax'}$), -70.1 (C$^7\underline{H}$), -72.3 (cyclen C$\underline{H}_{ax'}$); λ_{max} (H_2O) = 276 nm.

4-(4'-Nitropyridyl-2-methyl)-1, 7-bis(^{t}butoxycarbonylmethyl)-1, 4, 7, 10-tetraazacylododecane



(7-*tert*-Butoxycarbonylmethyl-1, 4, 7, 10-tetraazacyclododec-1-yl)-acetic acid *tert*-butyl ester (60 mg, 0.149 mmol) and potassium carbonate (21 mg, 0.149 mmol) were stirred in anhydrous acetonitrile (15 mL) under an $Ar_{(g)}$ atmosphere for 30 min. 2-Methane sulphanato-methyl-4-nitropyridine (24 mg, 0.103 mmol) was added. The mixture was heated to 40 ^oC for 3 h until formation of di-alkylated product was observed by LCMS. The reaction mixture was cooled, solids removed by vacuum filtration, and solvent removed under reduced pressure. This gave a light yellow oil that was taken on to the next stage without further purification; *m/z* (HRMS$^+$) 537.3402 $[M+H]^+$ ($C_{26}H_{45}O_6N_6$ requires 537.3401).

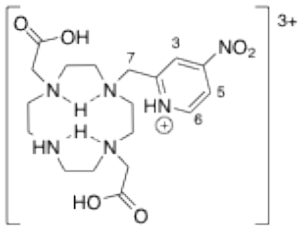
1-^{t}Butoxycarbonyl-4, 10-bis(^{t}butoxycarbonyl methyl)-7-(4'-nitropyridyl-2-methyl)-1, 4, 7, 10-tetraazacylodedecane



4-(4'-Nitropyridyl-2-methyl)-1, 7-bis(^{t}butoxy-carbonylmethyl)-1, 4, 7, 10-tetraazacylododecane (650 mg, 0.95 mmol) was dissolved in MeOH (10 mL). Di-*tert*-butyl dicarbonate (1.04 g, 4.75 mmol) was added and the mixture was stirred at r.t. for 18 h. The solvent was removed and the resulting residue was purified by silica gel column chromatography (gradient elution: 100 % CH_2Cl_2 to 6 % CH_3OH : CH_2Cl_2),

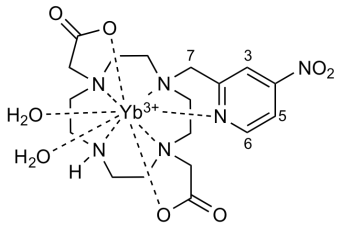
to give a yellow oil (210 mg, 35 %); ^{1}H NMR ($CDCl_3$, δ): 8.78 (1H, d, $^{3}J_{H\text{-}H}$ 3 Hz, H^6), 8.75 (1H, br, H^3), 7.86 (1H, d, $^{3}J_{H\text{-}H}$ 3 Hz, H^5), 3.87 (2H, br s, $2H^7$), 3.49 (4H, s, $C\underline{H}_2CO_2{}^{t}Bu$), 2.65 - 3.27 (16H, m, cyclen-$C\underline{H}_2$), 1.45 (9H, s, $CO_2{}^{t}\underline{Bu}$), 1.39 (18H, s, $CH_2CO_2{}^{t}\underline{Bu}$); *m/z* (HRMS^{+}) 637.3935 $[M+H]^{+}$ ($C_{31}H_{53}N_6O_8$ requires 637.3925). R_f = 0.43, 15 % CH_3OH : CH_2Cl_2.

Ligand $[H_3L^2]^{3+}$



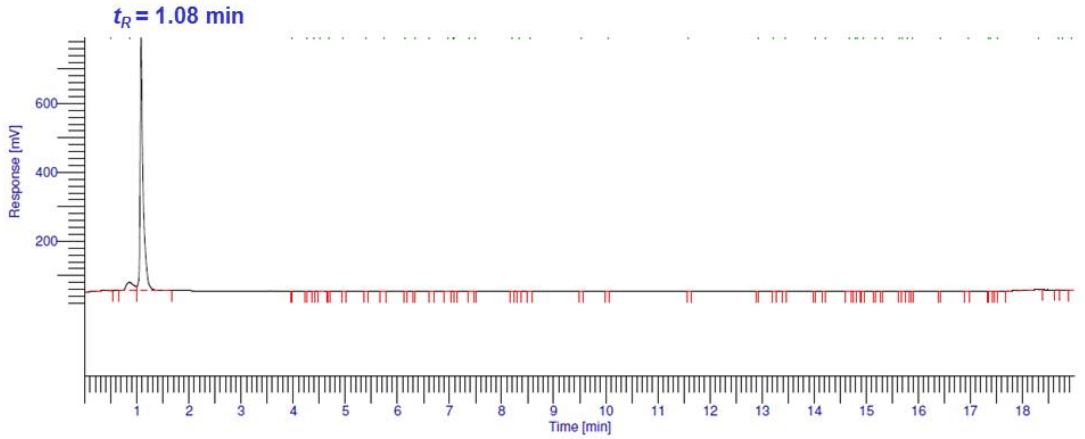
1-^{t}Butoxycarbonyl-4, 10-bis(^{t}butoxycarbonyl methyl)-7-(4'-nitropyridyl-2-methyl)-1, 4, 7, 10-tetraazacylododecane (70 mg, 0.109 mmol) was dissolved in CH_2Cl_2 (0.5 mL) and the mixture was cooled to 0 ^{o}C. TFA (1 mL) was added slowly, followed by water (0.2 mL), and the mixture was allowed to warm to r.t. and stirred for 24 h. The solvent was removed under reduced pressure to afford the tris(trifluoroacetate) salt of **L^2** as, a pale orange glass (83 mg, quantitative); ^{1}H NMR (D_2O, δ): 8.67 (1H, d, $^{3}J_{H\text{-}H}$ 5 Hz, H^6), 8.03 (1H, d, $^{4}J_{H\text{-}H}$ 2 H^3), 7.94 (1H, dd, $^{3}J_{H\text{-}H}$ 5 Hz, $^{4}J_{H\text{-}H}$ 2 Hz, H^5), 4.71 (2H, s, $2H^7$), 2.64 - 3.53 (20H, m, cyclen-$C\underline{H}_2$ and $C\underline{H}_2CO_2H$); *m/z* (HRMS^{+}) 425.2145 $[M+H]^{+}$ ($C_{18}H_{29}N_6O_6$ requires 425.2149).

[Yb.L^2]



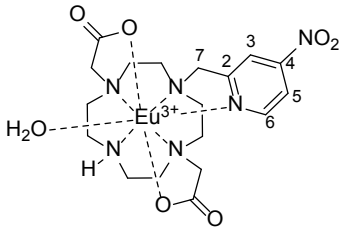
Complexation of ligand **L^2** (55 mg, 0.130 mmol) with Yb^{3+} using ytterbium (III) chloride hexahydrate (60 mg, 0.156 mmol) was undertaken as for [**Yb.L^1**]. The product was isolated as a white solid (90 mg, quantitative); decomposes above 200 ^{o}C; ^{1}H NMR (D_2O, δ): Spectral range of 165 ppm (+70 to -95 ppm); *m/z* (HRMS^{+})

592.1242 $[M+H]^+$ ($C_{18}H_{26}N_6O_6{}^{170}Yb$ requires 592.1262); analytical RP-HPLC: t_R = 1.08 min [gradient: 5 − 50% methanol in water (0.1% formic acid) over 15 min]; λ_{max} (H_2O) = 298 nm.

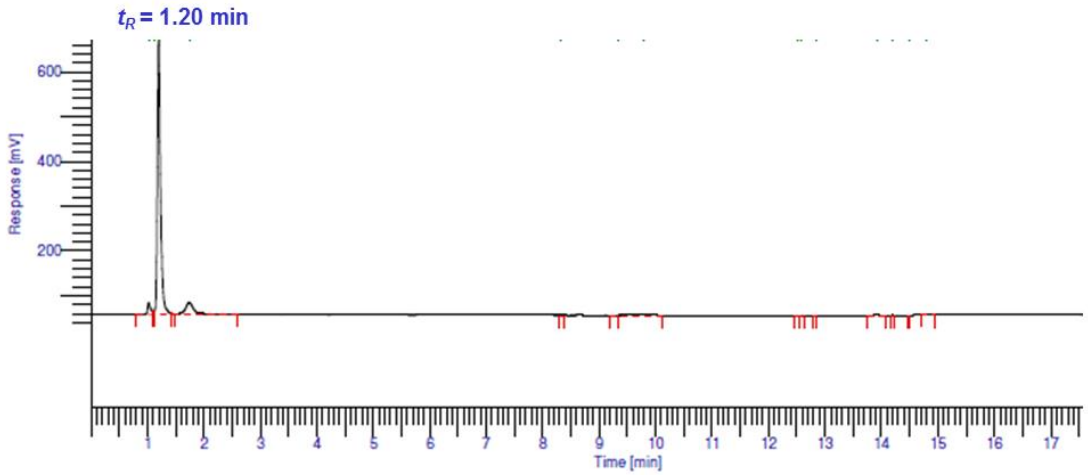


Analytical RP-HPLC trace of [**Yb.L²**]: t_R 1.08 min [Gradient: 5 to 50% methanol in water (0.1% formic acid) over 15 min]

[Eu.L²]

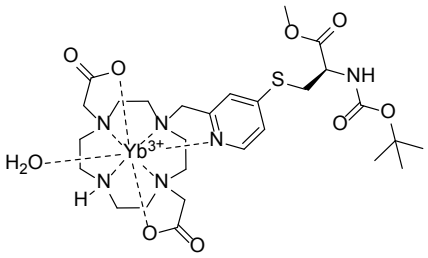


Complexation of ligand **L²** (18 mg, 0.0425 mmol) with Eu^{3+} using europium (III) chloride hexahydrate (19 mg, 0.0509 mmol) was executed as above for complex [**Yb.L²**]. The product was isolated as a white solid (30 mg, quantitative); decomposes above 180 °C; ^{1}H NMR (D_2O, δ): Spectral width of 81 ppm (+56 to -25 ppm); *m/z* ($HRMS^+$) 573.1102 $[M+H]^+$ ($C_{18}H_{26}N_6O_6S^{151}Eu$ requires 573.1112); Analytical RP-HPLC: t_R = 1.20 min [gradient: 5 − 50% methanol in water (0.1% formic acid) over 15 min]; λ_{max} (H_2O) = 298 nm ; τ_{H2O} = 0.43 ms; τ_{D2O} = 0.75 ms, q = 0.8.



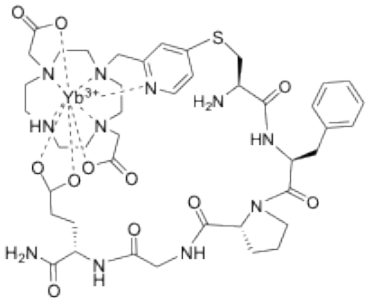
Analytical RP-HPLC trace of [**Eu.L^2**]: t_R 1.20 min [Gradient: 5 to 50% methanol in water (0.1% formic acid) over 15 min]

[Yb.L^2]-Boc-Cys-OMe



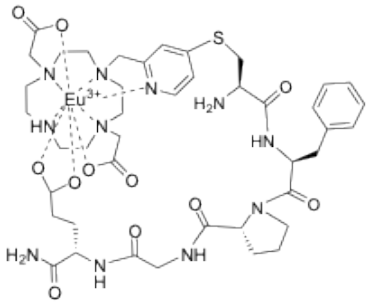
[**Yb.L^2**] (5 mg, 0.0084 mmol) was dissolved in DMF (1 mL) and *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (2.6 μL, 0.0126 mmol) and DIPEA (6.5 μL, 0.0252 mmol) were added and the mixture was stirred at r.t. for 18 h. The solvent was removed under reduced pressure to afford [**Yb.L^2**]-Boc-Cys-OMe as an off-white solid (6 mg, 91 %); m.p. 109-113 ^{o}C; ^{1}H NMR (D_2O, δ): spectral range of 183 ppm (+88 to -95 ppm); *m/z* (HRMS$^+$) 780.2148 $[M+H]^+$ ($C_{27}H_{42}N_6O_8S^{170}Yb$ requires 780.2133); λ_{max} (H_2O) = 280 nm.

[Yb.L^2-peptide 1]



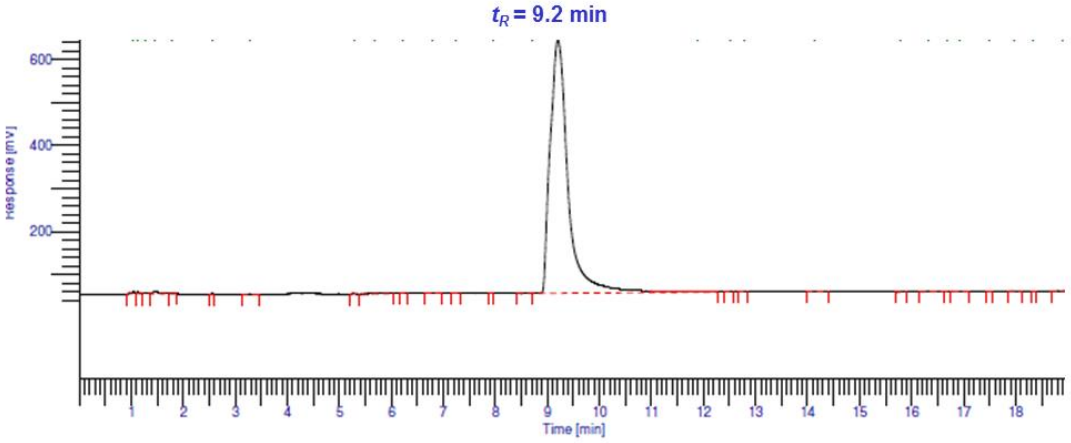
To a solution of [**Yb.L^2**] (5 mg, 8.4 μmol) in water (0.7 mL) was added the peptide NH_2-Cys-Phe-DPro-Gly-Glu-$CONH_2$ (5.2 mg, 8.4 μmol) and DIPEA (3 μL, 16.8 μmol), and the mixture was stirred at r.t. for 2 h. The solvent was removed under reduced pressure to give the conjugate [**Yb.L^2-peptide 1**] as a white solid (quantitative). Decomposes at 190 °C; ^{1}H NMR (D_2O, δ): spectral range of 197 ppm (+107 to -90 ppm); *m/z* (HRMS$^-$) 1093.332 $[M-H]^-$ ($C_{42}H_{57}N_{11}O_{11}S^{170}Yb$ requires 1093.331); analytical RP-HPLC: t_R = 1.22 min [gradient: 5 − 100% methanol in water (0.1% formic acid) over 15 min]; λ_{max} (H_2O) = 280 nm.

[Eu.L^2-peptide 1]



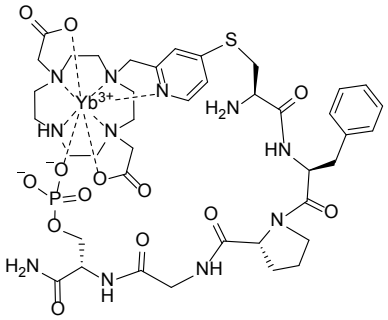
To a solution of [**Eu.L^2**] (3.8 mg, 6.2 μmol) in water (0.7 mL) was added **peptide 1**, NH_2-Cys-Phe-DPro-Gly-Glu-$CONH_2$ (2.8 mg, 6.2 μmol) and the pH of the solution was adjusted to 7.4 by the addition of KOH. The mixture was stirred at r.t. for 2 h, after which the solvent was removed under reduced pressure to give the conjugate [**Eu.L^2-peptide 1**] as a white solid (quantitative); m.p. 101-103 °C; ^{1}H NMR (D_2O,

δ): spectral range of 70 ppm (+45 to -25 ppm); *m/z* (HRMS$^+$) 1076.332 $[M+H]^+$ ($C_{42}H_{59}N_{11}O_{11}S$ ^{151}Eu requires 1076.332); analytical RP-HPLC: t_R = 9.2 min [gradient: 5 − 50% methanol in water (0.1% formic acid) over 15 min]; λ_{max} (H_2O) = 280 nm; $\tau_{H2O} = 0.42$ ms; $\tau_{D2O} = 0.60$ ms, q = 0.4.

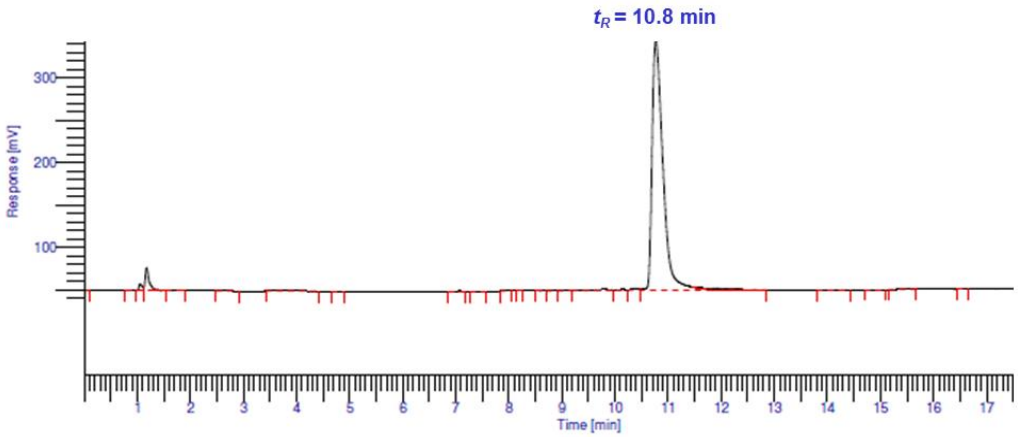


Analytical RP-HPLC trace of **[Eu.L^2-peptide 1]**: t_R 9.2 min [Gradient: 5 to 50% methanol in water (0.1% formic acid) over 15 min]

[Yb.L^2-Peptide 2]

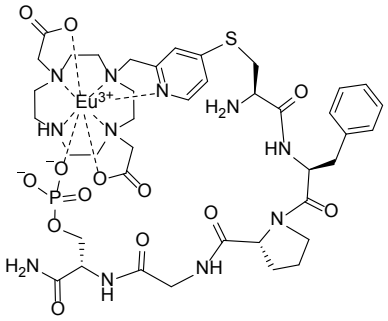


To a solution of **[Yb.L^2]** (7.6 mg, 12.8 μmol) in D_2O (0.6 mL) was added **peptide 2**, NH_2-Cys-Phe-DPro-Gly-pSer-$CONH_2$ (7.5 mg, 12.8 μmol) and DIPEA (4.5 μL, 25.6 μmol), and the mixture was stirred at r.t. for 2 h, after which the solvent was removed under reduced pressure to give the conjugate **[Eu.L^2-Peptide 2]** as a white solid (quantitative); Decomposes at 180 °C; ^{1}H NMR (CD_3OD, δ): spectral range of 200 ppm (+125 to -75 ppm); *m/z* (HRMS$^+$) 1134.301 $[M+H]^+$ ($C_{40}H_{57}N_{11}O_{13}PS^{171}Yb$ requires 1134.304); analytical RP-HPLC: t_R = 10.8 min [gradient: 5 − 50% methanol in water (0.1% formic acid) over 15 min]; λ_{max} (H_2O) = 276 nm.

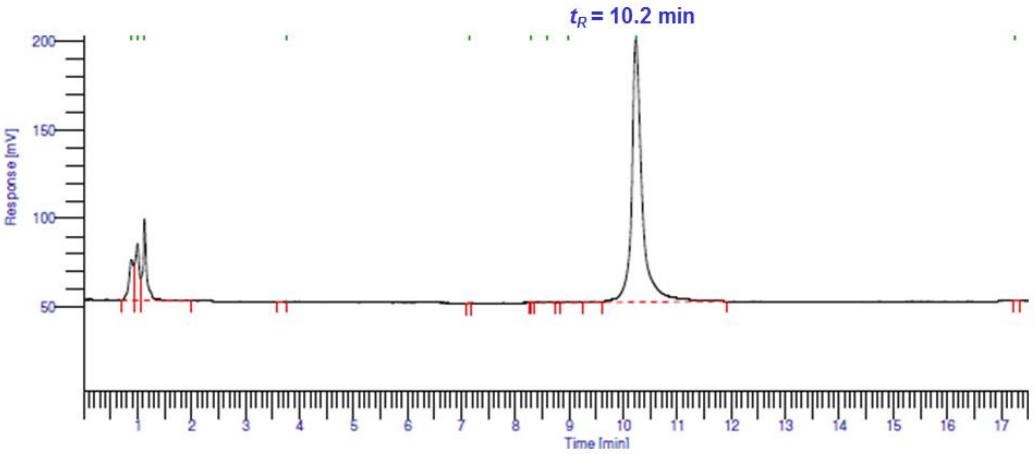


Analytical RP-HPLC trace of [**Yb.L^2-peptide 2**]: t_R 10.8 min [Gradient: 5 to 50% methanol in water (0.1% formic acid) over 15 min]

[Eu.L^2-Peptide 2]

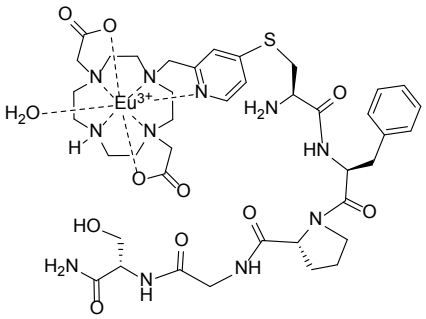


To a solution of [**Eu.L^2**] (1.5 mg, 2.6 μmol) in water (0.3 mL) was added **peptide 2**, NH_2-Cys-Phe-DPro-Gly-pSer-$CONH_2$ (1.5 mg, 2.6 μmol) and the pH of the solution was adjusted to 7.4 by the addition of KOH. The mixture was stirred at r.t. for 2 h, after which the solvent was removed under reduced pressure to give the conjugate **[Eu.L^2-Peptide 2]** as a white solid (quantitative); m.p. 110-112 ^oC; ^1H NMR (CD_3OD, δ): spectral range of 63 ppm (+43 to -20 ppm); *m/z* ($HRMS^+$) 1114.290 $[M+H]^+$ ($C_{40}H_{57}N_{11}O_{13}PS^{151}Eu$ requires 1114.287); analytical RP-HPLC: t_R = 10.2 min [gradient: 5 − 50% methanol in water (0.1% formic acid) over 15 min]; λ_{max} (H_2O) = 276 nm; τ_{H2O} = 0.46 ms; τ_{D2O} = 0.59 ms, q = 0.2.

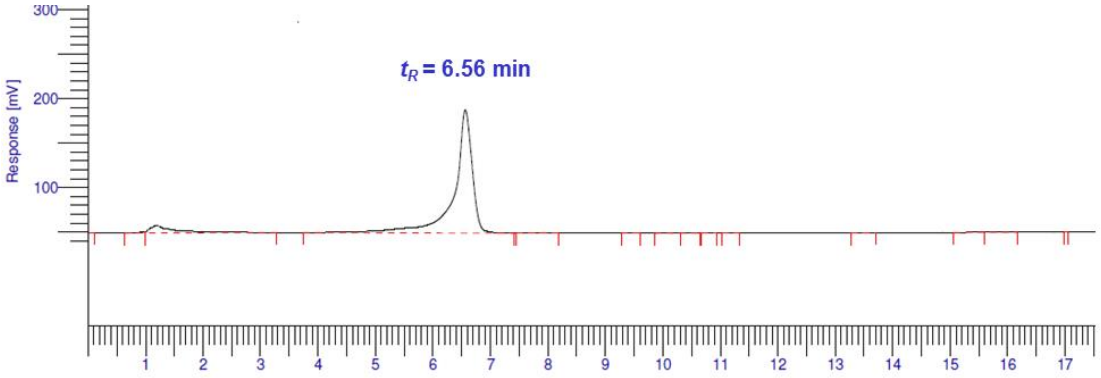


Analytical RP-HPLC trace of [**Eu.L^2-peptide 2**]: t_R 10.2 min [Gradient: 5 to 50% methanol in water (0.1% formic acid) over 15 min]

[Eu.L^2-Peptide 3]



To a solution of [**Eu.L^2**] (1.5 mg, 2.6 μmol) in water (0.3 mL) was added **peptide 3**, NH_2-Cys-Phe-DPro-Gly-Ser-$CONH_2$ (1.3 mg, 2.6 μmol) and the pH of the solution was adjusted to 7.4 by the addition of KOH. The mixture was stirred at r.t. for 2 h, after which the solvent was removed under reduced pressure to give the conjugate **[Eu.L^2-Peptide 3]** as a white solid (quantitative); m.p. 98-100 ^oC; ^1H NMR (CD_3OD, δ): spectral range of 67 ppm (+39 to -28 ppm); *m/z* (HRMS$^+$) 1034.322 $[M+H]^+$ ($C_{40}H_{57}N_{11}O_{10}S^{151}Eu$ requires 1034.321); analytical RP-HPLC: t_R = 6.6 min [gradient: 5 − 50% methanol in water (0.1% formic acid) over 15 min]; λ_{max} (H_2O) = 282 nm; $\tau_{H2O} = 0.48$ ms; $\tau_{D2O} = 0.94$ ms, q = 0.8.



Analytical RP-HPLC trace of [**Eu.L^2-peptide 3**]: t_R 6.6 min [Gradient: 5 to 50% methanol in water (0.1% formic acid) over 15 min]

Procedure for the modification of BSA to give [Dy.L^1]-derivatised BSA.

To a solution of [**Dy.L^1**] (1.0 mg, 1.6 mmol) in water (200 μL) was added a solution of bovine serum albumin (BSA, ultrapure crystals) (10 mg, 0.15 μmol) in water (200 μL) and the pH of the solution was adjusted to 7.4 by the addition of KOH solution. The mixture was stirred at rt for 1 h. The cysteine-modified BSA was analysed by MALDI-TOF MS, which revealed peaks at 66456 and 67059 Da corresponding to native BSA and the **Dy.L^1**-derivatised BSA respectively (nitro-displaced **Dy.L^1** m/z = 598). The trypsin digestion of both the native protein and **Dy.L^1**-derivatised BSA was undertaken by the addition of trypsin solution (200 μL, 1 mgmL^{-1} in 1 mM HCl), followed by stirring at 37 ^{o}C for 4 h. The resulting trypsin-digested mixtures were analysed by MALDI-TOF MS and Q-TOF high resolution mass spectrometry.

References

1. M. Tamura, Y. Urano, K. Kikuchi, T. Higuchi, M. Hirobe, and T. Nagano, *Chem. Pharm. Bull.*, 2000, **48**, 1514–1518.

2. Selected spectroscopic data for complex conjugates

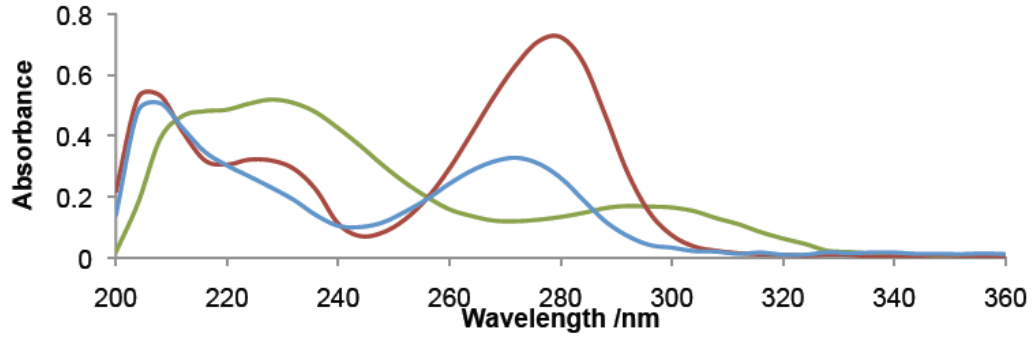


Figure S1. Overlaid absorption spectra showing: [**Yb.L^1**] (green); [**Yb.L^1**]-Boc-Cys-OMe (red); [**Yb.L^1**]-NH_2-Cys-OMe (blue), (293 K, H_2O, pH 6).

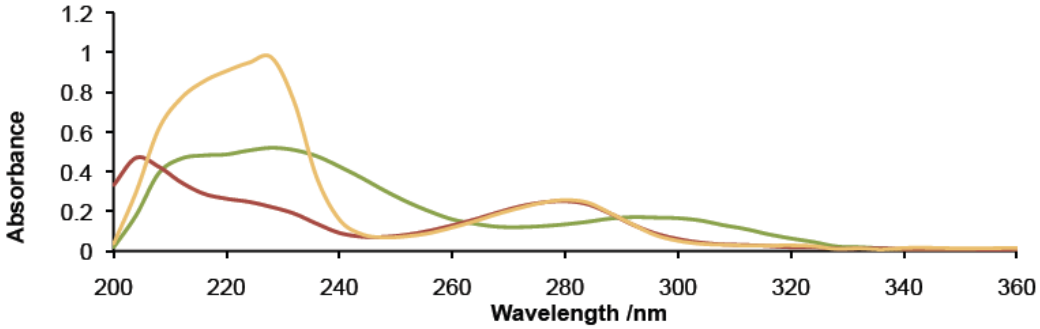


Figure S2. Overlaid absorption spectra showing: [**Yb.L^2**] (green); [**Yb.L^2**]-Boc-Cys-OMe (red); and [**Yb.L^2-peptide 1**] (orange), (295 K, H_2O, pH 6).

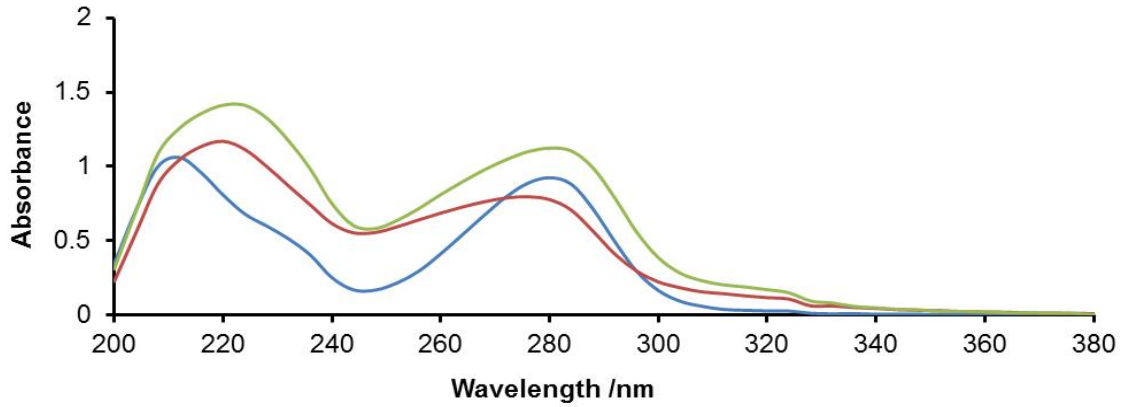


Figure S3. Overlaid absorption spectra showing: [**Eu.L^2-peptide 1**] (blue); [**Eu.L^2-peptide 2**] (red); and [**Eu.L^2-peptide 3**] (green), (295 K, H_2O, pH 6).

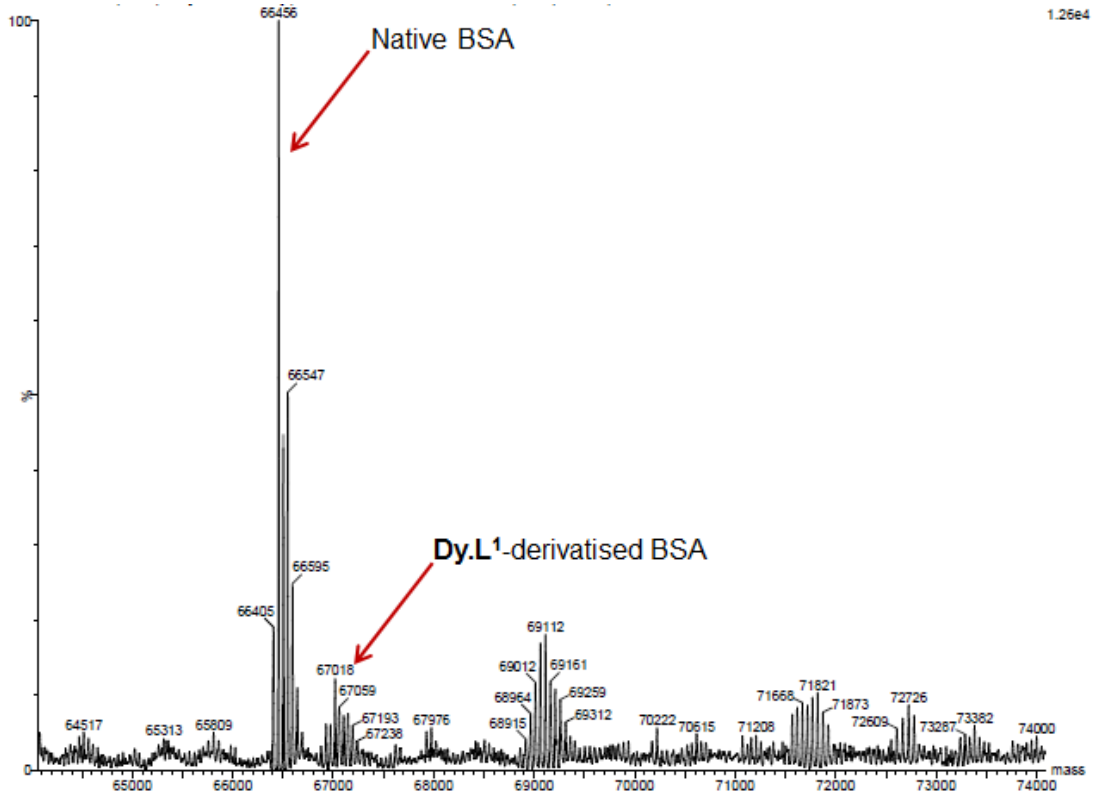


Figure S4. MALDI-TOF MS of the reaction mixture after incubation of bovine serum albumin (BSA) with [**Dy.L^1**] in water for 1 h. Highlighted are signals corresponding to both native BSA and **Dy.L^1**-derivatised BSA.

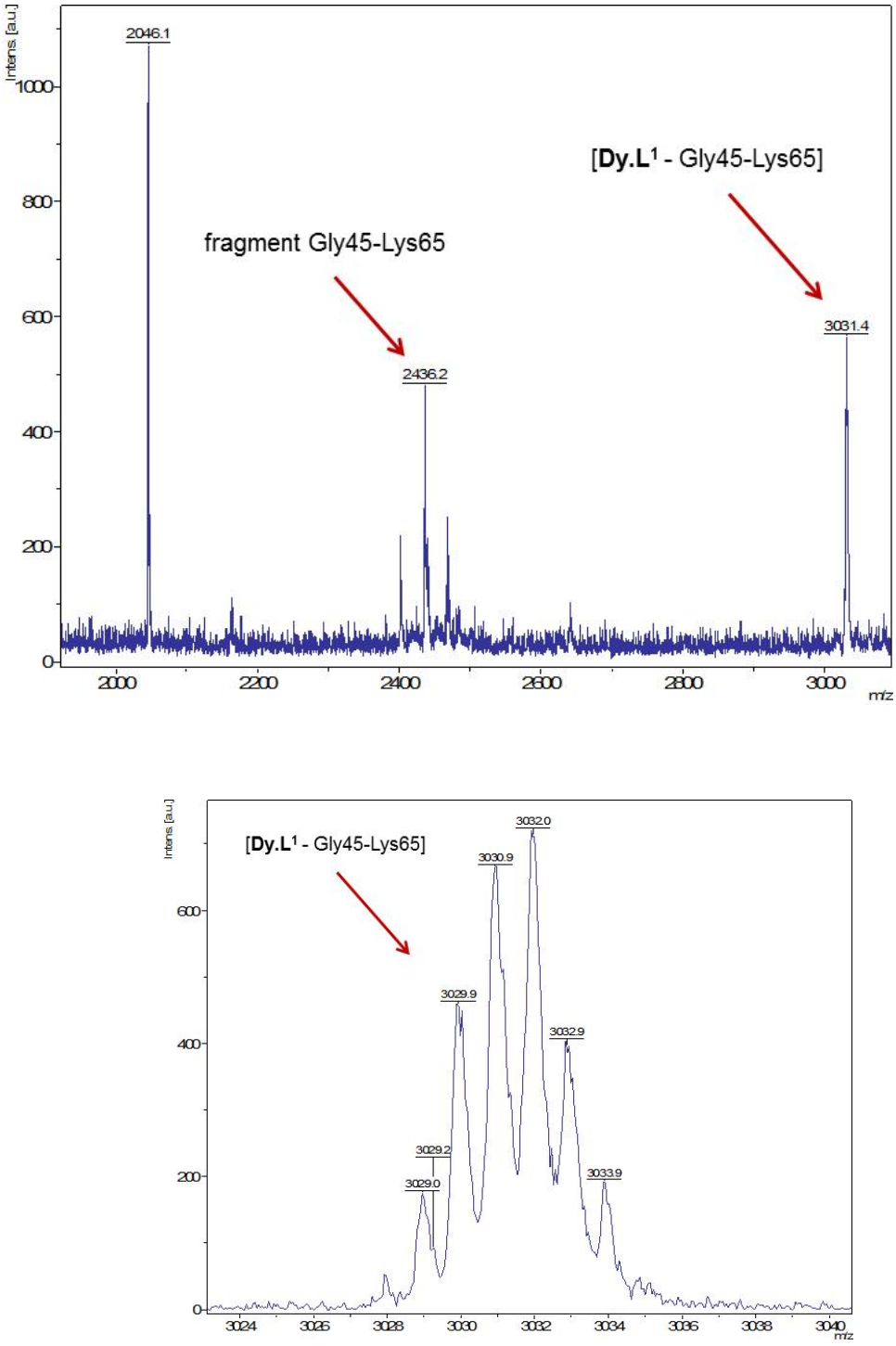


Figure S5. MALDI-TOF MS of the trypsin digest of **Dy.L^1**-derivatised BSA, revealing the peptide fragment Gly45-Lys65 $(GLVLIAFSQYLQQCPFDEHVK)^+$ and $[$**Dy.L^1** $–(Gly45\text{-}Lys65)]^+$. (*lower*) an expansion of the signal corresponding to $[$**Dy.L^1** $–(Gly45\text{-}Lys65)]^+$.

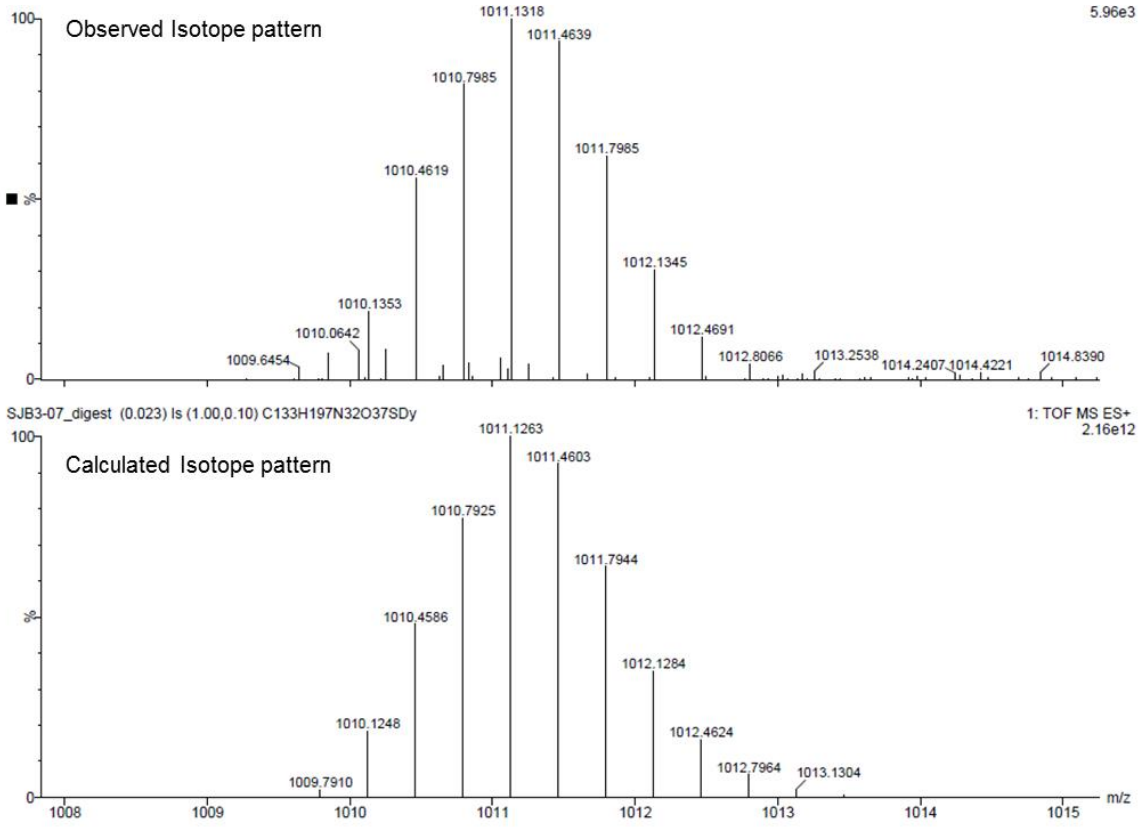


Figure S6. (*Upper*) Q-TOF High resolution mass spectrum ($HRMS^{+}$) of the trypsin digest of **Dy.L^{1}**-derivatised BSA, revealing the observed isotopic distribution of [**Dy.L^{1}**-(Gly45-Lys65)]$^{3+}$; (*lower*) calculated isotopic distribution for [**Dy.L^{1}**-(Gly45-Lys65)]$^{3+}$ ($C_{133}H_{198}N_{32}O_{37}SDy$).

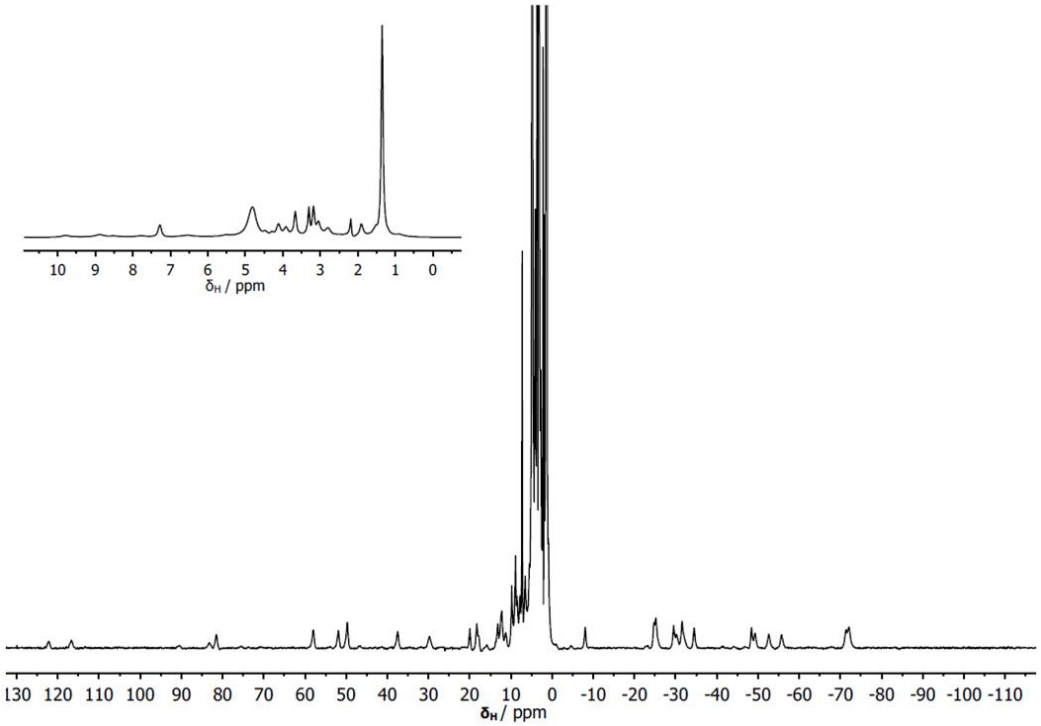


Figure S7. ^{1}H NMR spectrum of [**Yb.L^{2}-peptide 2**] recorded in CD_3OD (400 MHz, 295 K).

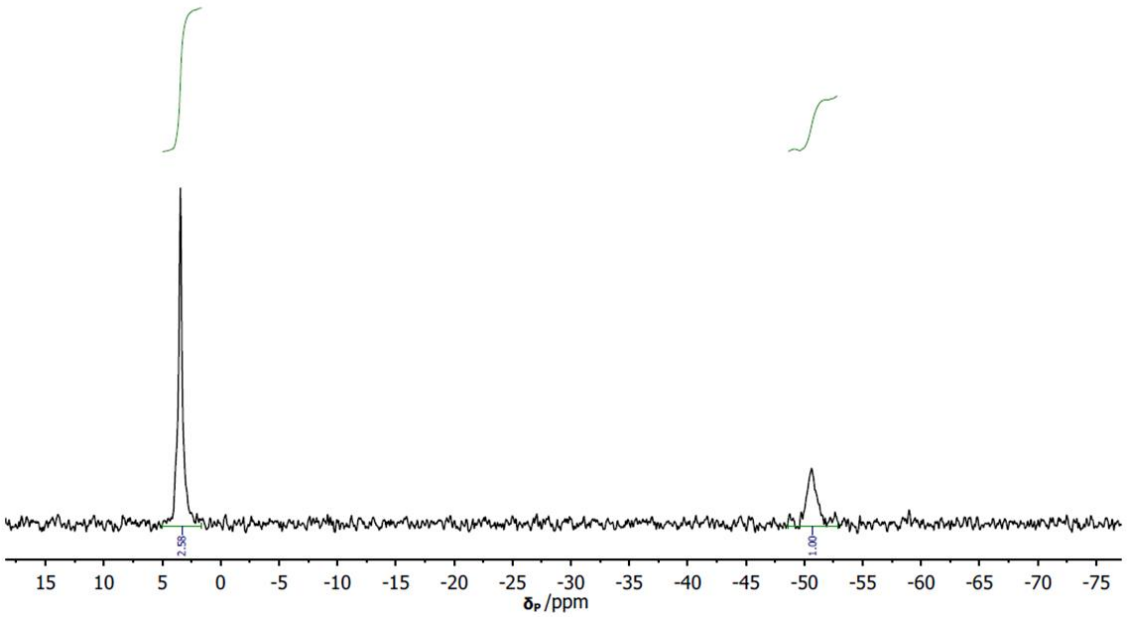


Figure S8. ^{31}P NMR spectrum of [**Yb.L^{2}-peptide 2**] recorded in CD_3OD (400 MHz, 295 K).

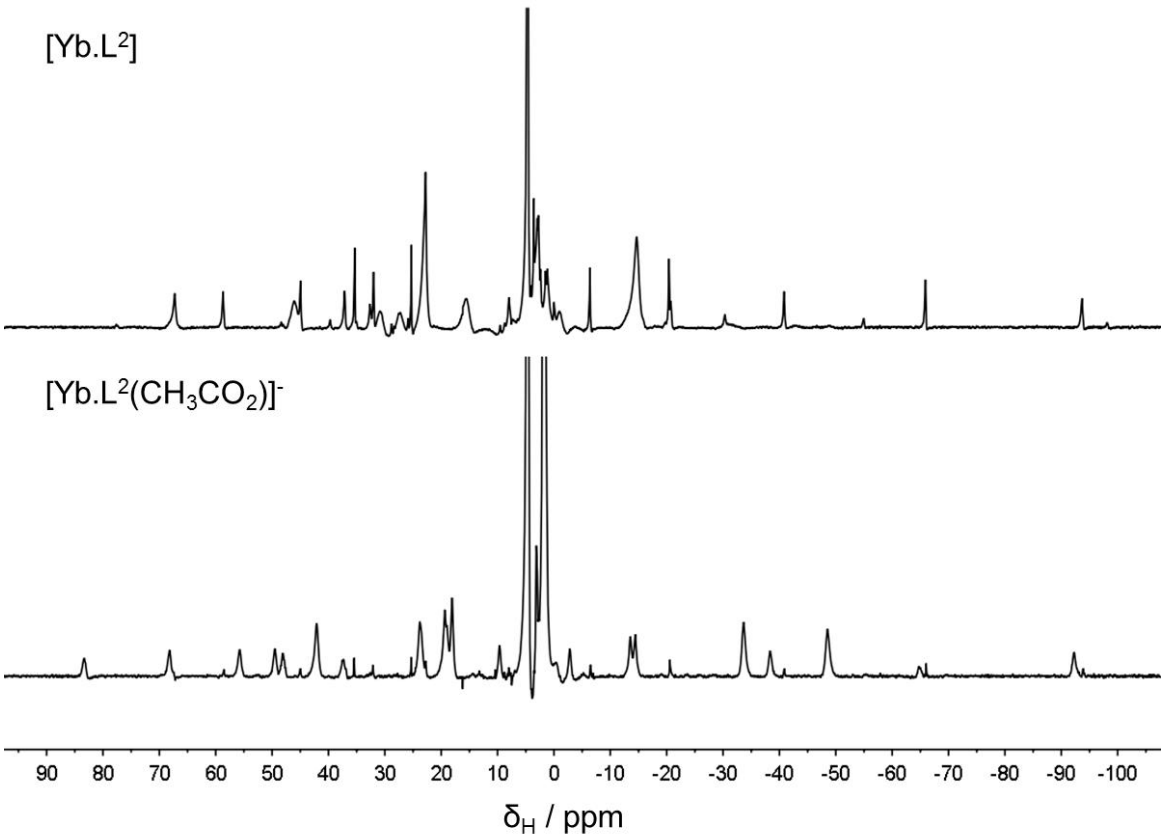


Figure S9. ^{1}H NMR spectra of: (*upper*) [**Yb.L^{2}**], and (*lower*) [**Yb.L^{2}**] in 1 mM NH_4OAc. Recorded in D_2O (400 MHz, 295 K).